

# Complete Genome of *Salmonella enterica* Serovar Typhimurium T5-Like Siphophage Stitch

James M. Grover,<sup>a</sup> Adrian J. Luna,<sup>a</sup> Thammajun L. Wood,<sup>b</sup> Karthik R. Chamakura,<sup>a</sup> Gabriel F. Kutyl Everett<sup>a</sup>

Center for Phage Technology, Texas A&M University, College Station, Texas, USA<sup>a</sup>; Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania, USA<sup>b</sup>

J.M.G. and A.J.L. are co-first authors.

**Salmonellosis, caused by *Salmonella*, is a leading cause of food poisoning worldwide. With the continuing rise of bacterial antibiotic resistance, efforts are focused on seeking new approaches for treatment of bacterial infections, namely, bacteriophage therapy. Here, we report the complete genome of *S. Typhimurium* siphophage Stitch.**

Received 1 December 2014 Accepted 18 December 2014 Published 5 February 2015

**Citation** Grover JM, Luna AJ, Wood TL, Chamakura KR, Kutyl Everett GF. 2015. Complete genome of *Salmonella enterica* serovar Typhimurium T5-like siphophage Stitch. *Genome Announc* 3(1):e01435-14. doi:10.1128/genomeA.01435-14.

**Copyright** © 2015 Grover et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Gabriel F. Kutyl Everett, [gabbyeverett@tamu.edu](mailto:gabbyeverett@tamu.edu).

**S**almonellosis is caused by serovars of the Gram-negative bacterium *Salmonella enterica* and is a leading cause of food poisoning (1, 2). With reports of antibiotic resistance on the rise, bacteriophage therapy presents an appealing alternative for treatment of bacterial infections (3, 4). Before a phage can be used in a clinical setting, much research is required to define various aspects of the phage's life cycle (5). To that end, we report here the complete genome of *S. enterica* serovar Typhimurium T5-like siphophage Stitch.

Stitch was isolated from a sewage sample collected in College Station, TX, USA. Phage DNA was sequenced using 454 pyrosequencing at the Emory GRA Genome Center (Emory University, GA, USA). Trimmed FLX Titanium reads were assembled to a single contig using the Newbler assembler, version 2.0.01.14 (454 Life Sciences), at default settings. Additional sequencing was performed at the Genomic Sequencing and Analysis Facility at the University of Texas (Austin, TX, USA) to give a single contig at 80.9-fold coverage. Genes were predicted using GeneMarkS (6) and corrected using software tools available on the Center for Phage Technology (CPT) Galaxy instance (<https://cpt.tamu.edu/galaxy-public/>). Transmission electron microscopy was performed at the Microscopy and Imaging Center at Texas A&M University.

Stitch shares 62% sequence identity with Enterobacteria phage T5 (NC\_005859), as determined by Emboss Stretcher analysis (7). The differences between the two phages occur largely in hypothetical conserved and novel genes. Stitch has a unit genome of 113,943 bp with 165 predicted coding sequences. A 9,982-bp-long terminal repeat was annotated using the PAUSE method (<https://cpt.tamu.edu/pause>) on raw sequencing data. Twenty-eight tRNA genes were identified in Stitch compared to the 16 tRNA genes present in T5.

As a T5-like phage, the genome of Stitch can be divided into several gene clusters. Proteins for host shutdown (presumably pre-early genes) were found, including 5'-deoxyribonucleotidase, A1, and A2 (8, 9). The early gene region encodes proteins whose

functions relate to DNA metabolism, replication, regulation, and lysis. The late gene cluster consists of morphogenesis genes. As with T5, a tail protein was found present in a noncanonical location among the major capsid protein, the prohead protease, portal, and large terminase (8). Stitch also encodes the lytic conversion lipoprotein (Llp). The Llp binds to host FhuA to prevent the lysed cell from inactivating released progeny (10, 11). Stitch contains no HNH homing endonucleases compared to T5's 9 homing endonucleases.

Stitch encodes a nicotinamide mononucleotide (NMN) transporter and an NMN adenylyltransferase that is found in T5-like phage EPS7 (NC\_010583) but not in T5 itself. NMN adenylyltransferase catalyzes the biosynthesis of NAD<sup>+</sup> and PPi from the condensation of NMN and ATP (12). As a T5-like phage, Stitch presumably has a nicked genome. It encodes A and B subunits of the NAD-dependent DNA ligase that are needed to seal the nicks and support genome replication (8). The NMN transporter and adenylyltransferase probably aids in efficient ligation and replication of the nicked genome.

**Nucleotide sequence accession number.** The genome sequence of phage Stitch was deposited in GenBank under the accession number [KM236244](https://ncbi.nlm.nih.gov/nucl/KM236244).

## ACKNOWLEDGMENTS

This work was supported primarily by funding from award EF-0949351, "Whole Phage Genomics: A Student-Based Approach," from the National Science Foundation. Additional support came from the Center for Phage Technology, an Initial University Multidisciplinary Research Initiative supported by Texas A&M University and Texas AgriLife, and from the Department of Biochemistry and Biophysics.

We are grateful for the advice and support of the CPT staff. This announcement was prepared in partial fulfillment of the requirements for Bich464 Phage Genomics, an undergraduate course at Texas A&M University.

## REFERENCES

1. Gole VC, Chousalkar KK, Roberts JR, Sexton M, May D, Tan J, Kiermeier A. 2014. Effect of egg washing and correlation between eggshell characteristics

- and egg penetration by various *Salmonella* Typhimurium strains. PLoS One 9:e90987. <http://dx.doi.org/10.1371/journal.pone.0090987>.
2. Hardy A. 2004. *Salmonella*: a continuing problem. Postgrad Med J 80: 541–545. <http://dx.doi.org/10.1136/pgmj.2003.016584>.
  3. Barton MD. 2014. Impact of antibiotic use in the swine industry. Curr Opin Microbiol 19:9–15. <http://dx.doi.org/10.1016/j.mib.2014.05.017>.
  4. Johnson RP, Gyles CL, Huff WE, Ojha S, Huff GR, Rath NC, Donoghue AM. 2008. Bacteriophages for prophylaxis and therapy in cattle, poultry and pigs. Anim Health Res Rev 9:201–215. <http://dx.doi.org/10.1017/S1466252308001576>.
  5. Keen EC. 2012. Phage therapy: concept to cure. Front Microbiol 3:238. <http://dx.doi.org/10.3389/fmicb.2012.00238>.
  6. Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes: implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. <http://dx.doi.org/10.1093/nar/29.12.2607>.
  7. Myers EW, Miller W. 1988. Optimal alignments in linear space. Comput Appl Biosci 4:11–17. <http://dx.doi.org/10.1093/bioinformatics/4.1.11>.
  8. Wang J, Jiang Y, Vincent M, Sun Y, Yu H, Wang J, Bao Q, Kong H, Hu S. 2005. Complete genome sequence of bacteriophage T5. Virology 332: 45–65. <http://dx.doi.org/10.1016/j.virol.2004.10.049>.
  9. Hendrickson HE, McCorquodale DJ. 1972. Genetic and physiological studies of bacteriophage T5: III—patterns of deoxyribonucleic acid synthesis induced by mutants of T5 and the identification of genes influencing the appearance of phage-induced dihydrofolate reductase and deoxyribonuclease. J Virol 9:981–989.
  10. Braun V, Killmann H, Herrmann C. 1994. Inactivation of FhuA at the cell surface of *Escherichia coli* K-12 by a phage T5 lipoprotein at the periplasmic face of the outer membrane. J Bacteriol 176:4710–4717.
  11. Decker K, Krauel V, Meesmann A, Heller KJ. 1994. Lytic conversion of *Escherichia coli* by bacteriophage T5: blocking of the FhuA receptor protein by a lipoprotein expressed early during infection. Mol Microbiol 12: 321–332. <http://dx.doi.org/10.1111/j.1365-2958.1994.tb01020.x>.
  12. Raffaelli N, Pisani FM, Lorenzi T, Emanuelli M, Amici A, Ruggieri S, Magni G. 1997. Characterization of nicotinamide mononucleotide adenyltransferase from thermophilic archaea. J Bacteriol 179: 7718–7723.